REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

Please consider this paper as a petition for a two-month extension of time. Please charge any required fees to have this Response entered to our deposit account No. 500687.

Claims 2-7, 11-14, 31-36 and 39-65 are pending in the application.

Basis for the amendment of claims 31 and 42, and for new claims 43 and 44 can be found in the originally filed application, including at pending claims 37 and 38. Basis for new claim 45 can be found at pending claim 35. Basis for new claims 46-65 can be found in previously pending claims 1-41. No new matter has been added.

Applicants reserve the right to pursue the subject matter of the original claims in a continuation application. All amendments have been made without prejudice.

Applicants' counsel thanks Examiner Tiffany M. Gough and Supervisory Examiner Jon Weber for the courtesy extended during the personal interview of 6 March 2008. The claim amendments set forth above and the arguments presented below are believed to be commensurate with the discussions held during the personal interview.

On page 2 of the pending Office Action, the Examiner refused to consider the foreign patents and applications submitted on 3/11/2004. Applicants again submit herewith copies of the foreign patents and applications. Applicants submit that the 3/11/2004 Information Disclosure Statement fully complies with the rules and full consideration of the listed documents is respectfully requested. Please charge any required fees to have the Information Disclosure Statement entered to our deposit account No. 500687.

The rejection of claims 1-7, 9-16, and 19-42 under 35 U.S.C. § 112, first paragraph, is obviated by the amendments to the claims as set forth above. The

Examiner argues on page 3 of the pending Office Action that:

> Claims 1-7, 9-16, 19-42 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically, the claims recite a method of enhancing enzymatic activity of an enzyme. Thus, the claims encompass the enzymatic enhancement of numerous potential enzymes, for which no written description has been provided. Moreover, the sole examples using alpha amylase and glucoamylase does not provide a representative sample of the enzymes encompassed by the claims, given the huge variation in characteristics, structural, and chemical properties encompassed by the current broad claim language. Because the claims encompass a multitude of enzymes neither contemplated nor disclosed by the as-filed disclosure, it is clear that applicant was not in possession of the full scope of the claimed subject matter at the time of filing.

All pending claims are directed to amylase or glucoamylase, which are clearly enabled by the experimental examples disclosed in the originally filed application. Accordingly, withdrawal of the Section 112 rejection is respectfully requested.

The rejection of claims 21, 31, 42 and their dependent claims 22-30 and 32-41 under 35 U.S.C. § 112, first paragraph, is respectfully traversed. On pages 3-4 of the Office Action, the Examiner argues that:

> Claims 21, 31, 42 and their dependent claims 22-30, 32-41 and its dependents are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically, the amendment of claims 21, 31 and 42 to read, "... if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;" introduces new matter, which is not described in the specification as originally filed. Claim 24, 34 requires that "the diluted enzyme exhibit at least the same level of enzyme activity per equal volume of the . . . ", this introduces new matter. Claim 25, 35 states

> that the solution is enhanced by at least 200%, this also introduces new matter. Claims 29, 41 requires that the pH of the solution maintains enzyme activity, this additionally introduces new matter.

Applicants respectfully submit that no new matter has been added by the claim amendments of record. In regards to claims 21, 31, and 42, the specification at page 2, lines 31-34 discloses that "[t]he crude enzyme extract may also be subjected to membrane separation, ion exchange, or ultrafiltration to produce a partially purified, concentrated enzyme extract rich in the desired enzyme, and relatively devoid of other competing/contaminating enzymes and/or cells." This language in the originally filed application clearly teaches that the enzyme solution can be filtered to remove the cells, i.e. to produce a solution devoid of cells. Applicants are not required to use the exact language recited in the original application. Thus, no new matter has been added in the amendment to claims 21, 31, and 42.

In regards to claims 24 and 34, the originally filed specification at page 4, lines 13-16 teaches that "[t]he raw enzyme solution is diluted with a desired amount of water or aqueous buffer solution for ease of mixing and separation of the activated carbon while, most surprisingly, at least maintaining its original level of enzymatic activity." This teaching provides clear support for claims 24 and 34, which recite "the diluted enzyme solution exhibits at least the same level of enzyme activity per equal volume of the undiluted enzyme solution." Thus, no new matter has been added.

In regards to claims 25 and 35, page 4, lines 10-16 of the original specification teaches that (1) the diluted enzyme has at least the same level of activity as the undiluted enzyme and (2) a minimum dilution factor of 3 times. A dilution factor of 1 is no dilution (0%), a dilution factor of 2 is doubling (100%) and a dilution factor of 3 is tripling (i.e. 200%). The dilution factor of 3 (200%) is the minimum, i.e. at least 200%. Thus, if a 200% dilution has the same level of activity as the undiluted enzyme after treatment according to the present invention, then "the activity of the enzyme solution is enhanced by at least 200%." The original application provides clear support for claims 25 and 35. Thus, no new matter has been added.

In regards to claims 29 and 41, page 1, line 26 of the originally filed application teaches that "[e]nzymes may be inactivated by extremes of temperature, pH ..." The specification also teaches to produce an active enzyme. See page 2, lines 11-19 of the present specification, which teach producing an active enzyme. Thus, combining these two teachings, the specification provides clear support for claims 29 and 41, which recite using a pH which maintains an "active" enzyme, not an inactive enzyme. Thus, no new matter has been added. Accordingly, withdrawal of the Section 112 rejection is respectfully requested.

The rejection of claims 1-7, 9-16, and 19-42 under 35 U.S.C. § 112, first paragraph, is obviated by the amendments to the claims as set forth above. On pages 4-6 of the Office Action, the Examiner states that:

> Claims 1-7,9-16,19-42 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the enhancement of enzymatic activity of the enzyme amylase, i.e. alpha and glucoamylase, does not reasonably provide enablement for enhancing the enzymatic activity of any and all enzymes by the claimed method. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. Specifically, as discussed above with respect to the issue of written description, the claims recite a method of enhancing enzymatic activity of an enzyme. The claims therefore encompass the enzymatic enhancement of numerous potential enzymes, for which no written description has been provided. Moreover, the sole examples using alpha amylase and glucoamylase does not provide a representative sample of the enzymes encompassed by the claims, given the huge variation in characteristics, structural, and chemical properties encompassed by the current broad claim. Given the property differences among enzymes such as structure, function and, one of ordinary skill in the art would not expect to be able to apply the disclosed method to any and all enzymes encompassed by the current claim language. Further, applicants own **arguments** support such lack of enablement. Page 12 of the arguments submitted 5/2/2007 states "because of structural and functional differences between proteins, an ideal sequence of steps for one protein will, quite possibly, be unsuccessful for another..." applicant also states on p.12, that "it is apparent that the procedure is not automatic-the fact that a procedure works provides no guarantee that it will work for another enzyme." Thus, with the exception of the above stated enzymes, and in view of the lack of

> any specific guidance with respect to the reaction conditions other than what is encompassed by the claims, one skilled in the art would expect a trial and error process to determine which enzymes encompassed by the claims would apply to the as disclosed application, and would further have to determine through undue experimentation, without guidance from the specification, how to perform such a method using any and all enzymes.
>
> Undue experimentation would be required to practice the invention as claimed due to the quantity of experimentation necessary to test each and every enzyme encompassed by the claim language, limited amount of guidance and limited number of working examples in the specification using a variety of enzymes; nature of the invention; state of the prior art; predictability or unpredictability in the art; and breadth of the claims. *In re Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).*

All pending claims are directed to amylase or glucoamylase, which are clearly enabled by the experimental examples disclosed in the originally filed application. Accordingly, withdrawal of the Section 112 rejection is respectfully requested.

Applicants respectfully submit that the Examiner unfairly takes Applicants' previous arguments out of context by arguing that "[f]urther, applicants own arguments support such lack of enablement. Page 12 of the arguments submitted 5/2/2007 states "because of structural and functional differences between proteins, an ideal sequence of steps for one protein will, quite possibly, be unsuccessful for another..." applicant also states on p.12, that "it is apparent that the procedure is not automatic-the fact that a procedure works provides no guarantee that it will work for another enzyme." Page 12 of Applicants' 2 May 2007 Response states:

> The Cornell lab manual for BIOBM330 discusses strategies for protein purification. (http://instruct1.cit.cornell.edu/Courses/biobm330/protlab/Strategy.html) The manual specifically states:
>
> > Because of structural and functional differences between proteins, an ideal sequence of steps for one protein will, quite possibly, be unsuccessful for another. A knowledge of the theoretical basis of each procedure will allow the researcher to choose an initial sequence of techniques with which to attempt any given purification. However, the development of an optimised protocol involves considerable

> trial-and-error experimentation to assess the potential of each step

The manual also specifically states:

> Note that the yield of enzyme after a particular purification procedure may be low not because the procedure is failing to purify that protein, but because it is causing some inactivation of the enzyme.

Based on these statements, it is apparent that the procedure is not automatic – the fact that a procedure works provides no guarantee that it will work for another enzyme. Furthermore, even maintenance of activity is difficult, and enhancement of activity is not automatic. [Emphasis added.]

It is quite clear the bolded language is referring to the known methods of purification, and not Applicants' novel method of enhancing the activity of enzymes. When this language is read in context with the entire 2 May 2007 Response and even the complete sentence, this language is saying that enhancement of activity is not automatic from the prior art purification methods and, in fact, "inactivation of the enzyme" is often seen in conventional purification methods. In no way are Applicants saying or even suggesting that the present invention does not work for all enzymes. Applicants do not even refer to the claimed method in the bolded sentences. Applicants request that this erroneous conclusion be withdrawn.

The rejection of claims 1-7 and 9-16 under 35 U.S.C. § 112, second paragraph, on page 6 of the Office Action, is moot in view of the amendments to the claims set forth above. Accordingly, withdrawal of the Section 11 rejection is respectfully requested.

The rejection of claims 1-7, 9-16, and 19-42 under 35 U.S.C. § 112, second paragraph, is respectfully traversed. On page 7 of the Office Action, the Examiner argues that:

> It is unclear what is meant by "raw enzyme and raw enzyme weight." Although, applicant defines "raw enzyme and raw enzyme weight" it is suggested that "crude enzyme" may better describe applicant's invention. Further, applicant fails to present "enzyme weight" in a standard

form of activity as is known in the art and as recommended by Methods of Enzymology. A clarification with respect to applicant's enzyme method is advised.

As admitted by the Examiner, the term "raw enzyme" is clearly defined on page 2, lines 26-36 of the originally filed specification as follows:

> By the term "raw enzyme solution" in this specification is meant a commercial grade formulation, produced by fermentation from any one of a variety of bacterial and microbial sources. In the case of an extracellular enzyme, the crude enzyme extract is obtained by, e.g., filtration or centrifugation of the fermentation broth, thus isolating the enzyme from protein debris. If the enzyme is produced intracellularly, the cells are lysed prior to filtration/centrifugation. The crude enzyme extract may also be subjected to membrane separation, ion exchange, or ultrafiltration to produce a partially purified, concentrated enzyme extract rich in the desired enzyme, and relatively devoid of other competing/contaminating enzymes and/or cells. The enzyme solution may also include residual components from the fermentation medium, protease inhibitors, and stabilizing agents.

Thus, the term "raw enzyme" clearly complies with Section 112. The term "enzyme weight" is clearly defined on page 3, lines 23-30 of the originally filed specification as follows:

> By the term "raw enzyme in connection with its weight" as used in this specification and claims is meant the volume of the raw enzyme solution x the density of the raw enzyme solution.
>
> The weight ratio of raw enzyme to purifying agent is dependent on the enzyme and purifying agent. Preferably, the ratio is not greater than 50:1, more preferably, not greater than 25:1, and still more preferably not greater than 15:1. A preferred ratio for use with activated carbon as the purifying agent provides 11g raw enzyme purified with 0.75 g activated carbon.

Thus, the claimed phrase "raw enzyme weight to activated carbon weight ratio" is clearly defined in the specification and fully complies with Section 112.

The Examiner argues on page 7 of the Office Action that "[c]laim 24 and 34 recites the limitation "undiluted enzyme solution". There is insufficient antecedent basis for this limitation in the claim." The term "undiluted enzyme solution" has clear basis on

page 6, line 28 of the originally filed application, which specification recites "undiluted enzyme." Reading claims 24 and 34 in light of the known meaning of the term "undiluted" and in light of the specification, the meaning of "undiluted enzyme solution" is quite clear, i.e. the starting enzyme solution before it is diluted according to the present invention. However, to further clarify this point, claim 34 has been amended to replace "the undiluted enzyme solution" with "the enzyme solution before dilution." The claim breadth has not been altered. No new matter has been added. Accordingly, withdrawal of the Section 112 rejection is respectfully requested.

Response to Prior Art Rejections:

Laustsen is the primary reference cited by the Examiner. Laustsen teaches a purification method that results in a 12% increase in flux. See page 2 of Laustsen's 2 December 2002 Response, courtesy copy attached. Laustsen does not provide any disclosure or even a suggestion of how to improve enzyme activity so that less amounts of enzyme are necessary. The experimental evidence now of record demonstrates that the claimed invention results in a surprising 200 to over 900% increase in enzyme activity. This experimental evidence rebuts any prima facie case of obviousness based on Laustsen, or any other reference that only teaches purification.

More specifically, within the originally filed patent application, the April 26, 2007 rule 132 declaration, the present rule 132 declaration, and the numerous articles of record, Applicants have conclusively demonstrated that:

1) Merely contacting undiluted commercial enzyme through activated carbon provides no measurable increase in enzyme activity.

2) Raw fermentation broth containing cells diluted by ~55% (approximately 1:1, the maximum taught by Laustsen) cannot be processed through activated carbon by the claimed method. The negligible permeability is due to the presence of cells in

the system, Applicants' use of high levels of activated carbon, and the fact that Applicants' process can be practiced under ambient pressure.

3) Raw fermentation broth which has cells removed and has been substantially diluted, e.g., 10-fold, or 1 part broth with 9 parts water/buffer, *can* be processed through the large quantity of activated carbon specified in the present patent application.

4) Commercial enzyme that has simply been diluted is well known in the art to have reduced activity, which is further confirmed by the experimental results in the present rule 132 declaration. In contrast, processing the diluted enzyme through activated carbon (as specified in the present patent application) leads to an unexpected significant (multi-fold) enhancement of activity.

5) Purification, as understood by those skilled in the art, does NOT automatically lead to an increase in enzyme activity. As demonstrated by the numerous references of record, a reduction in activity after purification is far more likely. Thus, purification methodologies are designed to minimize this well known adverse outcome

6) Purification, as understood by those skilled in the art, may lead to an undesirable change in structure and, thus, a change in CD or UV spectrum. This change in structure (UV and CD spectrum), as outlined in point (5), is known to be deleterious, and causes a REDUCTION in enzyme activity. Thus, conventional purification methodologies use structural analysis post-purification to ensure that structure has been PRESERVED, since this corresponds to retention of activity or minimizes the reduction of activity.

7) In an embodiment of the present invention, the interaction between the diluted purified enzyme and the activated carbon leads to a desirable structural change in the protein, as evidenced by a change in CD/UV spectrum and electrophoretic bands that is surprisingly not deleterious. Unlike conventional purification, this change in structure provides a heretofore unknown multi-fold INCREASE in activity. It is Applicants' hypothesis, without being bound by any theory, that this unexpected and substantial increase in activity arises from activated carbon's role as a catalyst and Applicants therefore refer to a catalytic or chemical change in protein structure, the result of which is an increase in activity. While activated carbon is known to have catalytic activity, it was unknown prior to the present invention that activated carbon could catalytically increase the activity of a purified and diluted enzyme, and surely not to such a large degree, up to 1,000%.

The present invention is distinct from Laustsen because the present invention requires the combination of: (a) removal of cells (purification) and Laustsen does not, (b) much higher levels of dilution (at least three-fold, and preferably 5-fold or 10-fold, while Laustsen cites, at most, a 1.1-fold dilution, to 45% of the original protein concentration), and (c) a high ratio of activated carbon to enzyme to provide the claimed enhancement of enzyme activity.

Applicants note that Laustsen discloses in paragraph 46 that "[a]ccording to the present invention the added amount of carbon is preferably from 0.05 to 2% (w/w) of the initial fermentation broth volume, in particular the added amount of carbon is from 0.1 to 1% (w/w) of the initial fermentation broth volume." At the maximum of 2%, this is a 50:1 range of fermentation broth to activated carbon. However, Laustsen clearly teaches to use amounts of activated carbon in a direction away from the claimed invention, i.e. far less activated carbon. First, Laustsen's preferred range is 0.1 to 1%, which at 1% is a 100:1 ratio. Second, all of Laustsen's working Examples 1-3 teach far less activated carbon, i.e., 0.2%, which is a 500:1 ratio. Furthermore, the attached Rule

132 Declaration demonstrates that even at low activated carbon amounts, 500:1, the process of Laustsen did not work unless pressure was applied and, thus, the much higher amounts of carbon claimed (not greater than 50:1) would cause further problems in the process of Laustsen, as discussed in the Rule 132 Declaration and below. In contrast, in the claimed invention the ratio of broth to carbon is not greater than 50:1. Note that a ratio of 49:1 has more activated carbon than a ratio of 50:1. Applicants further point out that the claimed activated carbon ratios are used in combination with other claimed steps, such as at least a 3 fold dilution and filtering the cells before contacting with activated carbon for a time sufficient to effect enzyme enhancement, which combination is not taught by Lausten.

All of the prior art rejections rely upon Laustsen (US2002/0020668). Applicants submit herewith a Rule 132 Declaration that clearly demonstrates the differences between Laustsen and the claimed invention.

The starting materials used in Example 2 of Laustsen are not all commercially available. Thus, Applicants contracted a fermentation facility to produce Laustsens' alpha amylase in accordance with the British patent 1,296,839 cited by Laustsen,, and then attempted to process the raw fermentation broth according to the conditions and ratios described by Laustsen in Example 2. For comparison, Applicants then also performed experiments using the fermentation broth diluted to 10% of its original concentration (a dilution ratio according to the present invention), and Applicants also performed experiments with an essentially cell-free enzyme preparation, following the claimed invention. Experiments were performed with the low level of activated carbon specified by Laustsen, and with the high level of activated carbon specified in claimed invention. Details of the experiments and the experimental observations are described in the Rule 132 Declaration.

Applicants original plan was to test the activity of the enzyme processes according to the procedure described by Laustsen. However, when Applicants ran the process according to Laustsen, none of the enzyme eluted, even though a more porous filter was used, so Applicants did not have a sample to test. Increasing the amount of

activated carbon, as per the present invention, did not improve the situation. Applicants were only able to collect samples from trials where the conditions were dramatically modified from those described by Laustsen, e.g., (1) by using much more diluted fermentation broth (1 part broth in 9 parts water, versus 1 part broth plus ~1 part water, as per Laustsen), (2) removing the cells first, as per the present invention, or (3) using a highly porous filter cloth. Even in the case of method (3), once the cells accumulated on the surface, Applicants could not collect much enzyme from the broth. Ultimately, these experiments confirmed that the claimed process is dramatically different from that described by Laustsen, and that cell removal and significant pre-dilution are key prerequisites to using the claimed process.

Furthermore, Applicants have demonstrated that large quantities of activated carbon are a disadvantage when the goal is filtration (Laustsen), but Applicants own work has also shown that large quantities of activated carbon are essential if the goal is activity enhancement. This further distinguishes the claimed method from Laustsen's. Since Laustsen's stated goal is filtration improvement, Laustsen cannot teach the use of large quantities of carbon, because it adversely affects filtration rate (flux). Without sufficient carbon (or retention time in the carbon), it is difficult to detect any change in enzyme activity if any actually occurs. Conversely, the present invention must use high levels of activated carbon, otherwise, Applicants do not get the claimed enhancement of enzyme activity. Too little activated carbon is a disadvantage for the present invention, whereas too much activated carbon is a disadvantage for Laustsen and, thus, Laustsen teaches in a direction away from the claimed invention. Given the claimed invention's distinct objectives, the operating conditions, dilution levels, and carbon loading must be distinctly different from the process of Laustsen.

Laustsen is owned by Novozymes, which is one of largest producers of enzymes in the world. Thus, Novozymes is highly skilled in the art of producing enzymes. From an economic perspective, given the significant shortage of fermentation capacity in the U.S., and the high cost to produce and ship enzymes, it would be enormously surprising if Novozymes failed to adopt the presently claimed technology if they found an activity

enhancement from their work with the Laustsen application. The present invention conservatively reduces enzyme costs by 10 to over 90% in a multi-billion dollar industry. Certainly, Laustsen, as a Novozymes employee, would therefore have primarily emphasized and claimed any results of enhanced activity, rather than results of enhanced permeation, if enhanced activity had been observed. Instead, the obvious conclusion is that enhanced activity was neither detected, nor expected by Laustsen. As noted above, to achieve their goal of enhanced microfiltration, Laustsen has to use small amounts of activated carbon to avoid clogging the filter, insufficient to create the activity enhancement seen (or possible) with the activated carbon loadings used in the presently claimed process. Thus, Laustsen actually teaches in a direction away from the claimed invention.

More specifically, the Rule 132 Declaration confirms that:

(1) A raw fermentation broth, even when diluted by ~55% as specified by Laustsen, cannot flow through the column. Even with very low quantities of activated carbon (i.e., 0.3g per 310 mL of diluted fermentation broth (1050:1 ratio)), there is no permeation through the column and column frit even though the frit utilized had a pore size of 20μm, which was far larger than Laustsen's disclosed pore size of 0.45μm. Thus, Laustsen's microfiltration process is dramatically different from the claimed method, which uses the combination of contact with high levels of activated carbon for a time sufficient to effect enzyme enhancement, much higher dilutions, and an essentially cell-free solution. Furthermore, Laustsen applies pressure across the membrane in order to achieve the stated fluxes, whereas the claimed invention is not limited to any particular pressure.

(2) If the porosity restriction imposed by the column frit is removed in Laustsen, e.g., by replacing the column frit with filter cloth, some very limited permeation of the diluted fermentation broth is observed, but the rate drops off rapidly once the cells accumulate within the activated carbon. Operating with the high level of activated carbon specified in the present application restricts the flow even further. Thus, the fermentation broths discussed by Laustsen cannot be processed using the level of

activated carbon specified by the present invention, even if the fermentation broth is diluted by ~55% as specified by Laustsen. If the broth is diluted to 10 times its original volume (a dilution rate specified by present invention), permeation is more rapid for a while, until the cells collect on the filter cloth, after which the permeation slows dramatically again.

(3) If a cell-free enzyme solution is used according to the claimed invention, the resulting diluted enzyme is readily permeable through the column, whether with the original column frit or with the filter cloth. If the filter cloth is used, the diluted enzyme passes through the column in a matter of seconds. This shows the need for a nearly cell-free solution and the need for a significantly diluted enzyme in order to process through the amount of activated carbon specified by claimed invention.

Also provided in the Rule 132 Declaration are the test results of a large scale production run using several hundred litres of a diluted enzyme solution produced according to the present invention. This work, conducted in 2003/04 in a 20 million gallon per year ethanol plant, demonstrates that the claimed modified alpha amylases surprisingly matched the performance of the industry standard alpha amylase (Liquozyme, from Novozymes) when added to the liquefaction stage of the ethanol plant. These experiments were based on an 80%/20% blend of the presently claimed modified enzymes with Liquozyme. The resulting sugar profiles, fermentation profiles, and flow data showed that the resulting blend was bioequivalent to the 100% Liquozyme, **in spite of the fact that the blend contained 75% less raw alpha amylase**. Clearly, the claimed process was instrumental in dramatically increasing the activity of the alpha amylase. Based on the specified addition rate of 65 mL/min, a 75% reduction in alpha amylase translates into a volumetric savings of around 2100 Litres per month, and thousands of dollars in savings to the ethanol plant.

The Rule 132 Declaration also provides a comparison between the mere dilution of a purified commercial enzyme compared to a diluted purified commercial enzyme that has been processed according to the claimed invention to provide enhanced enzyme activity. The experimental results conclusively demonstrate that the diluted

enzyme processed according the present invention exhibited a surprisingly far greater activity than the merely diluted enzyme.

This new experimental evidence, in combination with experimental evidence of record, both in the originally filed application and the 26 April 2007 Rule 132 Declaration of record, fully rebut any prima facie case of obviousness raised by the Examiner. Accordingly, withdrawal of all of the Section 103 rejections of record are respectfully requested.

The rejection of claims 1-6,9,10,19-23,27,28,29,31-33,37,39,40,42 under 35 U.S.C. § 103(a) as being unpatentable over Laustsen et al (US2002/0020668 Al) is respectfully traversed. The claimed invention is not obvious over Laustsen for the following reasons.

On pages 8-10 of the Office Action, the Examiner argues that:

> Applicant claims a method of enhancing the intrinsic activity of an enzyme solution, preferably a hydrolase such as amylase, glucoamylase and cellulase, by treating with a purifying agent, activated carbon. The raw enzyme solution is diluted with wither [sic] water and removed by filtration. Such method may also be carried out through a column. The enzyme to carbon ratio is not to exceed 50:1, preferably 15:1. Additionally that if any cells are present in the solution, that they are filtered out.
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> Laustsen teach the use of activated carbon in a fermentation broth, particularly with enzymes such as amylases and cellulases, to remove soluble impurities by purification improving the quality of a product (see abstract and 0015,0018-0046). The carbon is added at concentration of up to 2% w/w (see 0046). The enzyme solutions are further diluted with water before addition of carbon and further microfiltered (see examples 1 and 2), although they also teach the purification of such enzyme solutions with activated carbon may also be performed by such methods such as ultrafiltration, chromatographic methods, i.e. column method, adsorption and/or crystallization (see 0057). Laustsen also teaches that the fermentation broth may be treated prior to the method by separating out solids by filtration, flocculation or centrifugation, i.e. removing cells if present (0043 and 0044).
>
> Although the above references do not specifically state the enhancement of the enzyme activity, the method of treating a diluted enzyme solution with a purifying agent, activated carbon, is the same. Laustsen does not teach the exact dilution amounts and ratios. However, differences in concentration or temperature will not support the

> patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."). See MPEP 2144.05

Applicants note that the Examiner correctly points out that "differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical." The experimental evidence disclosed in the Rule 132 Declarations of record fully demonstrate the unexpected advantages of the claimed invention, which are directly attributable to the claimed steps and "differences in concentration." These unexpected advantages fully rebut any prima facie case raised by the Examiner.

Furthermore, Applicants submit that Laustsen teaches a very different method than the claimed method, and even teaches in a direction away from the claimed invention, for the reasons of record and, thus, Laustsen's method cannot be optimized to arrive at the claimed invention. Nevertheless, any optimization arguments are now mute in view of the overwhelming experimental evidence of record directly comparing the claimed invention to the teachings of Laustsen.

In view of the many differences between the claimed invention the cited prior art, and the many unexpected advantages of the claimed invention, withdrawal of the Section 103 rejection is respectfully requested.

The rejection of claims 1-6,9,10,12,13,15-17,19-23,27,28,29,31-33,37,39,40,

and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laustsen et al (U52002/0020668 Al) in view of Shenoy et al (J. of Bioscience, vol 7, 1985) and http://www.aplab.com/circular dichroism.htm is respectfully traversed. The claimed invention is not obvious over the theoretical combination of cited references for the many reasons of record and for following reasons.

The experimental evidence disclosed in the Rule 132 Declarations of record and discussed above fully rebut any prima facie case obviousness. For this reason alone the Section 103 rejection should be withdrawn.

The Examiner argues on pages 10-12 that:

> Applicant claims a method of enhancing the intrinsic activity of an enzyme solution by treating with a purifying agent. The enzyme solution of enhanced activity is claimed to have a relative absorbance intensity lower than the raw enzyme solution, preferably in the CD spectral range of 205-230 nm. Applicant further claims the enzyme to be alpha-amylase.
> As stated above, Laustsen teach the use of activated carbon in a fermentation broth, particularly with enzymes such as amylases and cellulases, to remove soluble impurities by purification improving the quality of a product (see abstract and 001 5,001 8-0046). The carbon is added at concentration of up to 2% w/w (see 0046). The enzyme solutions are further diluted with water before addition of carbon and further microfiltered (see examples 1 and 2), although they also teach the purification of such enzyme solutions with activated carbon may also be performed by such methods such as ultrafiltration, chromatographic methods, i.e. column method, adsorption and/or crystallization (see 0057). Laustsen also teaches that the fermentation broth may be treated prior to the method by separating out solids by filtration, flocculation or centrifugation, i.e. removing cells if present (0043 and 0044).
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> Laustsen does not teach a CD spectral ranges. Shenoy et a! (J. of Bioscience, vol7,1985) teach the purification of glucoamylases. They teach that the catalytic activity of a protein, i.e. enzyme is related to its "active" conformation, i.e. secondary and tertiary structure. The specific activity of the purified enzymes was three times higher than that of the original non-purified glucoamylase (see p.400). They teach that the UV (CD) spectra of glucoamylases from 3 species show peaks at 289-293,279-282 and 257-259 nm (see p.400-402), but also reveal negative bands at 217-220,208-210 (see p. 402).
>
> Shenoy does not teach lower CD spectrum ranges such as those claimed by applicant nor alpha-amylase. Information found at

> http://www.aplab.com/circular_dichroism.htm teaches that any change in structure of proteins will affect the CD spectral range, therefore a change in the spectral range appears to be an inherent property of purification, i.e, structural change, of a protein. Thus, one of ordinary skill in the art would be motivated and it would therefore be obvious to claim a CD spectral range lower than that of a raw enzyme solution given that a change in structure ultimately affects the CD spectrum. When purifying a protein such as enzymes, one would have a reasonable expectation of success in obtaining a CD spectrum range lower than that of the raw enzyme solution given that purification ,enhancing the catalytic activity of an enzyme, ultimately alters the secondary and tertiary structure, therefore altering the CD spectrum range. Further, it would be obvious to optimize these parameters through routine experimentation.
>
> Also it would be obvious to use other hydrolase enzyme such as alpha-amylase because Laustsen teach the use of activated carbon in a fermentation broth, particularly with enzymes such as amylases and cellulases, to remove soluble impurities by purification improving the quality of a product (see abstract and 0015,0018-0046). Therefore, one of ordinary skill in the art at the time of the invention would have been motivated to purify an enzyme such as alpha-amylase with activated carbon as taught by Laustsen and would have a reasonable expectation of success in obtaining a CD spectral range lower than that of the raw enzyme solution given what is known in the art of the change in structure by purification of a protein.

As argued previously, claims 11 to 15 recite **changes in the defined spectra,** not specific CD ranges. In other words, the spectra for the claimed enzyme solution is different from the conventional enzyme solution in the recited spectral range, which is very different from claiming a specific spectral range. In fact, in contrast, the goal of purification is to *avoid* a structural (and spectral) change, as demonstrated by the following statement in instructional materials from Cornell University: "Purification procedures attempt to maintain the protein in native form. Although some proteins can be re-natured, most cannot!"

The Examiner states that "any change in structure of proteins will affect the CD spectral range". This is indeed true, but Applicants respectfully submit that the Examiner's subsequent conclusion that "a change in spectral range appears to be an inherent property of purification" is incorrect. As noted in a multitude of other texts and

references on protein purification discussed in Applicants previous 2 May 2007 Response, the goal is to *preserve* structure, because this is the most effective way to preserve activity. Changes in structure are, in most instances, correlated with loss of activity, due to chemical or thermal denaturation. Tools such as circular dichroism and x-ray crystallography are thus used to examine protein structure, ostensibly to determine if purification has had an adverse impact on protein structure.

In an authoritative review on the use of circular dichroism, Kelly and Price (Curr. Protein and Peptide Sci., 1, 349-384, 2000) state (page 373) that CD is used to "address the question of the retention of native structure on extraction, purification and crystallisation." Thus, CD is used after the fact, to ensure that the native structure has not been altered by extraction, purification, or crystallization. Additional supporting references are included in the Appendix attached to Applicants 2 May 2007 Response.

An embodiment of the claimed process is unique in that it leads to a change in protein structure, which is believed, without being bound by any theory, to be due to catalytic modification of the protein, that enhances the specific activity of the protein, unlike a traditional purification process that simply acts to concentrate a protein. In a traditional purification process, there is an inevitable reduction in (active) protein, but an even greater reduction in volume, resulting in a product solution that is more concentrated in the desired protein. In the present process, Applicants have effected an increase in the volume of enzyme solution to provide a less concentrated protein (due to dilution), while increasing the enzyme activity, in some cases even maintaining the baseline activity of the original (already purified) enzyme solution. This multi-fold enhancement in enzyme activity per unit mass of protein is believed to be due to catalytic transformation of the protein, changing its structure, and leading to a change in its spectral properties as measured by CD and by UV.

Consequently, the Examiner's statement that "a change in the spectral range appears to be an inherent property of purification" is inconsistent with widely-held knowledge about the process of protein purification, since an ideal purification procedure would preserve protein structure, and avoid a change in spectral properties.

In view of the many differences between the claimed invention and the unexpected advantages of record, withdrawal of the Section 103 rejection is respectfully requested.

The rejection of claims 1-6,9,10,11,1419-23,27,28,29,31-33,37,39,40,42 under 35 U.S.C. 103(a) as being unpatentable over Aikat et al (Biotechnology Letters, vol 23, 2001 p.295-301) in view of Laustsen et al (U52002/0020668 Al). The claimed invention is not obvious over the theoretical combination of cited references for the reasons of record and for the following reasons.

The experimental evidence now of record in two Rule 132 Declarations rebuts any case of prima facie obviousness for the reasons provided above. For this reason alone, the Section 103 rejection should be withdrawn.

The Examiner argues on pages 12-14 of the Office Action that:

> Applicant claims a method of enhancing the intrinsic activity of an enzyme solution, specifically a hydrolase, by treating with a purifying agent, activated carbon, and further removing the activated carbon from the enzyme solution by centrifugation. The purified enzyme solution is said to have a CD and UV spectrum distinct from that of the raw enzyme solution, specifically 30 nm less and the enzyme to carbon ratio is not to exceed 15:1.
>
> Aikat et al. teach the purification of protease by activated charcoal, i.e. activated carbon. They demonstrate the purification by activated charcoal in terms of fold purification and by electrophoretic analysis (see introduction). The enzyme solution was mixed with activated charcoal and allowed to react for a specific period of time prior to centrifugation, thus removing the activated carbon, at which time the supernatant was examined by spectroscopy. Further analysis was carried out by electrophoresis (see p. 296). The enzyme solution (1 ml) was treated with 50 to 150 mg of activated charcoal, although 75 mg of charcoal was selected as their optimum ratio. By gel analysis they observed the removal of almost all of the smaller proteins, confirming the purifying action of activated charcoal.
>
> Further, Aikat diluted the crude enzyme solution 10 times to bring its absorbance within the range of that of charcoal-treated enzyme, which shows distinct troughs at 260 nm and a peak at 280nm. In the crude diluted solution there appeared to be a peak at 260 nm and no valley (see p.299 to 300).
>
> Aikat does not teach diluting the raw enzyme solution prior to

> treating with a purifying agent. However, as stated above Laustsen teach diluting an enzyme solution prior to treating the activated carbon. Thus , at the time of the invention it would have been obvious to one of ordinary skill in the art to dilute an enzyme solution prior to treatment because the prior art teaches dilution prior to treatment with activated carbon. Moreover, at the time of the claimed invention, one of ordinary skill in the art would have been motivated to have diluted an enzyme solution prior to purification with activated carbon with a reasonable expectation for successfully enhancing the intrinsic activity of such solution because the art teaches such success when using the claimed enzyme solution and purifying agent.

The experimental evidence demonstrates that merely contacting the enzyme solution with activated carbon does not result in an increase in enzyme activity. See the graphs in the 26 April 2007 Rule 132 Declaration of record, center bars (no dilution, contact with activated carbon), which are the same height as the left most bars representing the original enzyme solution. In contrast, when the enzyme solution is diluted with an aqueous solution, cells are removed when present, and then contacted with the claimed amount of activated carbon, an astonishing 10 fold (1,000%) increase in enzyme activity is obtained. See the graphs in the Rule 132 Declaration, right most bars of each three bar set. If the enzyme activity did not increase 10 fold, the right most bars would be 1/10 the height of the left most bars for each set of three bars. For this reason alone the Section 103 rejection should be withdrawn.

The Examiner has acknowledged that Aikat does not teach diluting the raw enzyme solution prior to treatment with a activated carbon. Applicants respectfully submit that one of ordinary skill in the art would <u>not</u> have been motivated to dilute an enzyme solution prior to purification with activated carbon with a reasonable expectation for successfully enhancing the intrinsic activity of such solution, because the art <u>does not</u> teach such success when using the claimed enzyme solution or any other enzyme solution and activated carbon.

Furthermore, Laustsen does not teach the combination of the claimed steps of diluting to a level of at least 3 times, removing cells if present, contacting with activated carbon for a time sufficient to form an enzyme solution having enhanced activity, and

removing the activated carbon. As discussed previously, Laustsen teaches diluting an enzyme broth containing cells only to a level of about 2.2 times (110%) and contacting the solution containing cells to carbon. None of the cited references, alone or in any combination, teach the claimed steps of dilution at least 3 times (200%), removing cells if present, and then contacting the diluted solution with activated carbon in an amount and for a time to effect enhancement of the enzyme activity. For this reason alone, the Section 103 rejection should be withdrawn.

The cited references do not teach or suggest the unexpected results of the claimed invention. The key to the results of Aikat is their electrophoresis results. The images of the crude protein and the charcoal-treated proteins show that the smaller proteins were almost completely removed by the activated carbon. This is clearly a quintessential case of protein purification, not dilution and enhancement according to the present invention.

By comparison, an embodiment of the claimed process results in an extra band when glucoamylase is treated, and the smaller proteins are more abundant when amylase is treated (shown as A, A', B, B' in Figure 3). The observations are consistent with a structural change in the native protein, but not consistent with the simple purification described by Aikat. It can therefore be concluded that Aikat have performed a simple purification – small proteins, debris, and extracellular nucleic acids have been removed by adsorption and/or size exclusion, while leaving the treated protein unaffected. In contrast, the claimed process leads to transformation of the enzyme and dilution accompanied by enhanced activity.

There is nothing within Laustsen to suggest an increase in activity arising from treatment with activated carbon and the Examiner previously acknowledged that Laustsen does not "specifically state the enhancement of enzyme activity." See page 5 of the 10 January 2007 Office Action.

The Examiner now simply concludes that "at the time of the claimed invention, one of ordinary skill in the art would have been motivated to have diluted an enzyme solution prior to purification with activated carbon with a reasonable expectation for

successfully enhancing the intrinsic activity of such solution because the art teaches such success when using the claimed enzyme solution and purifying agent." The art absolutely does ***not*** teach that the enzyme activity can be enhanced, as admitted previously by the Examiner, and surely not by an astounding 200 to 1,000%.

Indeed, if the Examiner's conclusion was correct, previously cited Bailey should have seen an increase in activity when they contacted enzyme with activated carbon during immobilization, but they did not – in fact, in their patent, they explicitly state that there was no benefit observed when some types of activated carbon were used in their process. See Applicants previous Response. Furthermore, the Rule 132 Declaration dated 26 April 2007 absolutely confirms that merely contacting the enzyme with activated carbon does not result in enhanced enzyme activity.

In view of the many differences between the claimed invention and the cited prior art, and the unexpected advantages of record, withdrawal of the Section 103 rejection is respectfully requested.

The Examiner argues on pages 14-15 of the Office Action that:

> Applicants 132 Declaration filed 5/2/2007 fails to show evidence commensurate in scope with the present application. Specifically, applicant has done a comparison, in Fig.1 and 2, of a native enzyme solution, the enzyme solution processed through activated carbon, and that of amylase produced by the method claimed, i.e. dilution and processing through an activated carbon column. It is unclear how one could conclude that such a graph and comparison would provide unexpected results commensurate in scope with applicant's invention. Applicant also does not provide how such activity is being measured, i.e. specific activity measured in the units as known in Enzymology. Applicant should do a side-by-side comparison of the diluted enzyme without being processed with activated carbon. There is something to be said of an enzyme solution which has been diluted and it's endogenous inhibitors affecting an increase after dilution, i.e an inhibitor which possesses a low affinity for the enzyme. Changes in activity or inhibition with dilution are a function of the specific enzyme and amount of enzyme in the initial enzyme solution. Further, activated carbon is a known absorbent, therefore the effect may be explained by the fact that the small molecules present in solution are inhibitors of the enzymes therefore binding to the activated carbon, allowing a more pure enzyme to remain. The offices position is that a more effective comparison may include additionally the

> same diluted enzyme solution not processed with activated carbon compared to the same volume, amount of enzyme diluted solution which has been processed with activated carbon. It is unclear from the Figure legends in applicants declaration which boxes correspond to the white and grey shaded bars.

Even if the Examiner's comments regarding removing inhibitors is correct, there is no explanation for the change of structure, as demonstrated by the CD spectra. Furthermore, the cited art does not teach a large enhancement of enzyme activity (200 to >900%), using the Examiner's hypothesis of removing inhibitors by filtration.

Applicants respectfully submit that the experimental evidence submitted in the Rule 132 Declaration dated 26 April 2007 is commensurate in scope with the claimed invention. As correctly pointed out by the Examiner, "applicant has done a comparison, in Fig.1 and 2, of a native enzyme solution, the enzyme solution processed through activated carbon, and that of amylase produced by the method claimed, i.e. dilution and processing through an activated carbon column." Thus, Applicants have compared "amylase produced by the method claimed" to the actual prior art, i.e. a "native enzyme solution" and native "enzyme processed through activated carbon." These test results were conducted at the request of the Examiner to compare the claimed invention to the prior art, specifically native enzyme solutions and enzyme solutions processed through activated carbon as taught in the cited references. Thus, the Rule 132 Declaration is commensurate in scope in comparing the claimed invention to the cited prior art.

The Examiner now requires Applicants to compare the claimed invention to a diluted native enzyme that has not been processed with carbon. As requested, Applicants submit herewith experimental evidence in the attached Rule 132 Declaration directly comparing the claimed invention (dilution of purified enzyme and processing with activated carbon in an amount to effect an increase in enzyme activity) to a merely diluted purified enzyme. This new experimental evidence conclusively confirms what is already known in the art, mere dilution substantially reduces enzyme activity.

The Examiner also argues that it "is unclear from the Figure legends in applicants declaration which boxes correspond to the white and grey shaded bars." In

Figures 1 and 2 of the 26 April 2007 Declaration, each time interval has three bars, a left bar, a center bar, and a right bar, and a legend is shown in a box in the upper left hand portion of the Fig. In each Fig., the uppermost legend ("Avg. Brix (liquozyme SC DS)" for Fig. 1) represents the left bar, with the second legend corresponding to the center bar, and the bottom legend to the right bar.

In response to the Examiner's question regarding how the enzyme activity is measured, Applicants used the well known Brix method. Brix is a measurement of the refractive index of a solution. Since the refractive index of sugars differs from that of water, the production (or consumption) of sugars can be monitored by measuring the Brix of a solution, and observing how it changes over time. Thus, the Brix values shown in Figures 1 and 2 of the 26 April 2007 Rule 132 Declaration are measured values of the (aggregate) sugar concentration in the solution. Since sugar production is directly correlated to the activity of the enzyme, the Brix measurements in Figs. 1 and 2 are, therefore, accurate indicators of enzyme activity. See paragraph 6 in the Rule 132 Declaration submitted herewith, which confirms that the Brix method was utilized in the 26 April 2007 Declaration.

On pages 15-16 of the Office Action, the Examiner argues that:

> Applicant arguments in response to the Laustsen reference have been considered, however, applicant argues that Laustsen discloses an enzyme dilution of 1:1, while this is true, they also disclose at least a two part dilution, see example 2, which teaches at least 2 parts. Applicant also does not show in the submitted Declaration that a dilution of 2:1 is better than or has an unexpected increase of activity compared to a 1:1 solution. Applicant also argues that Laustsen dilutes an enzyme broth which contains cells and in contrast applicants invention contains a broth in which the cells have been filtered out first. Applicant does not specifically claim whether or not the cells are present and/or if they have been filtered out. Thus, applicants arguments are not commensurate in scope with the claimed invention. Applicant argues that the Declaration submitted on 5/2/2007 teaches that filtering an enzyme solution through an activated carbon column does not inherently increase enzyme activity. However, as stated above, the showing in the declaration does not accurately show or compare "dilution, removal of cells if present, and then contacting with the activated carbon" which results in a surprising enhancement of activity (see p.11 of Arguments). Applicant merely compares the native enzyme

solution, the solution with activated carbon and that which has been diluted. Therefore, the arguments are not persuasive.

Applicants respectfully submit that Laustsen does not teach a 2 part dilution in Example 2 as alleged by the Examiner. A 2 part dilution is a 200% increase in volume, i.e. starting with 1 liter and then adding 2 more liters (2 part or 200% dilution) to arrive at a total of 3 liters. Example 2 of Lausten specifically teaches starting with about 150 liters (150kg) of raw fermentation broth, to which 160 liters of water is added (amount of water to dilute to 310 liters total) and then adding about 6.9 liters (6.9kg) of $Na_2Al_2O_4$ for a total overall amount of about 317 liters of liquid. Thus, Laustsen at best teaches adding 167 liters to a starting amount of 150 liters, which is a 1.1 part (110%) dilution—far less than the minimum claimed 2 part (200%) dilution.

The Examiner argues that "the showing in the declaration does not accurately show or compare "dilution, removal of cells if present, and then contacting with the activated carbon" which results in a surprising enhancement of activity (see p.11 of Arguments). Applicant merely compares the native enzyme solution, the solution with activated carbon and that which has been diluted." This simply is not true.

Applicant compared the claimed invention, a 10 fold (1,000%) diluted enzyme solution (which is free of cells) processed with an amount of activated carbon to provide enhanced activity, to the cited prior art teachings (1) undiluted enzyme and (2) undiluted enzyme solution merely contacted with activated carbon. Furthermore, Applicants have now compared the claimed method (dilution of purified enzyme processed with amount of activated carbon to effect enhanced enzyme activity) with a merely diluted enzyme in the presently filed Rule 132 Declaration, which conclusively confirms that mere dilution does not result in enhanced enzyme activity. The present Rule 132 declaration demonstrates that the claimed invention provided a surprisingly far greater enzyme activity compared to the enzyme activity of the merely diluted enzyme. Applicants request that the Examiner consider the experimental evidence of record and withdraw the prior art rejections of record.

On page16 of the Office Action, the Examiner argues that:

> It is noted that applicant has submitted many documents teaching away from applicants claimed invention, i.e. attempting to overcome the Offices inherency rejection and to show unexpected results.
>
> Regarding applicant's arguments directed towards the Shenoy reference, i.e. that Shenoy does not teach purification resulting in activity three times higher than the original non-purified glucoamylase, rather compared to a parent strain. While this has been considered, applicant does not specifically claim nor show in the Declaration, purification of an original non-purified enzyme. Applicant actually compares in the declaration dated 5/2/2007, an already purified commercial enzyme to a diluted enzyme purified by the claimed method. Thus, applicant's arguments are not commensurate in scope with the present invention.

Applicants' claimed invention *requires* purification, i.e. removal of cells, **only if cells are present** before application of the activated carbon. Thus, if an already purified enzyme solution is utilized in the claimed invention, then the step of removing cells is not required since they are already removed. In either case, a purified enzyme solution free of cells is enhanced according to the claimed steps. An already purified enzyme can absolutely be used to practice the claimed invention.

The novelty of Applicants' invention is that the enzyme activity can be greatly enhanced, surprisingly greater than 200%, by the claimed steps of diluting an enzyme solution (which has been purified by removing cells) with an aqueous solution by at least 3 times, and then processing with an amount of activated carbon and for a time sufficient to effect an enhancement of enzyme activity. As correctly pointed out by the Examiner, the examples used purified enzyme, i.e. no cells present. The step of removing cells if present does not alter this experimental evidence, since the cells are absent either way. The enzyme activity of the purified enzyme was conducted according to the claims steps and, thus, the experimental evidence is commensurate in scope with the claimed invention and must be considered.

The cited prior art does not teach or suggest the claimed steps. Furthermore, the prior art does not teach or even suggest the surprising 200% to 1,000% increase in enzyme activity.

Applicants respectfully submit that the Examiner improperly cites the teachings of Shenoy by assuming that the 3-fold increase in activity is due to purification. Shenoy

does not explicitly state that the enhancement of activity is due to purification. Based on the general knowledge in the art, as represented by the numerous references now of record, purification mostly results in reduced enzyme activity and at best the same enzyme activity. Thus, there is no support in art or Shenoy for the Examiner's position that Shenoy teaches enhanced activity can be obtained by purification. While the wording in Shenoy is vague, one of ordinary skill in the art would properly interpret the meaning of Shenoy's statements that **the enzyme activity enhancement is due to genetic modification of the organism**, rather than due to purification.

In the second part of the Examiner's argument, reference is made to the May 2, 2007 Rule 132 Declaration, wherein "purification" of an already purified enzyme is discussed. To be clear, the Declaration shows activities of enzymes before and after they have been processed using the presently claimed technology. The activity of the untreated enzyme, which is a commercially available purified enzyme, has been compared to the activity of this same enzyme after it has been subjected to the claimed process. The Examiner appears to believe that the similarity in activity is due to the fact that the commercial enzyme has already been purified before Applicants have received it, and so, further "purification" by Applicants is moot. However, the Examiner has not taken into account the fact that the claimed processed enzyme has **1/10th the protein** (i.e., enzyme) as the original, unprocessed enzyme. The fact that Applicants can accomplish this dramatic increase in enzyme activity on an already purified enzyme further supports the claimed invention. Simple removal of inhibitors and other "non-essential" proteins would have already been largely accomplished during the manufacturer's purification step, which thus rules out the Examiner's other hypothesis that enhancement is due to removal of these inhibitors. To see a further 10-fold improvement in activity after Applicants have processed an already purified enzyme is therefore unexpected and fully rebuts the prior art rejections of record.

On pages 16-17 of the Office Action, the Examiner argues that:

> Applicant's arguments directed to spectral change continue to be confusing. Applicant is arguing that it is the goal of purification to avoid a structural and spectral change, yet claim a spectral change, which is

> distinct from the "raw enzyme solution", as in claim 11,12,13,30. The argument is contradictory. The examiner is well aware of the fact that CD is used after the fact to determine alteration in the structure. Applicant argues that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. Shenoy also teach that the catalytic activity of a protein, i.e. enzyme is related to its "active" conformation, i.e. secondary and tertiary structure. They state that the ideal purification would preserve protein structure and avoid change in spectral properties, yet claim that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. This argument is not understood.

Applicants' arguments are not confusing to one of ordinary skill in the art. As accurately stated, the goal of purification is to avoid a structural and spectral change. This is a well known fact, which is supported by the numerous references now of record and discussed fully in Applicants' 2 May 2007 Response.

Applicants' invention is not mere purification. The claimed invention recites steps that are not described in the prior art and are not mere purification. As stated previously and confirmed by the experimental evidence of record, the claimed invention results in a dramatic and unexpected increase in enzyme activity. As a proposed explanation, without being bound by any theory, Applicants also submitted the structural and spectral data.

In a typical purification process, as understood by those of ordinary skill in the art, the goal is to avoid a change in structure, so that activity is mostly preserved. The claimed process is not typical, and in fact, might not even be classified as purification, because an embodiment of the claimed process appears to cause a change in structure, and the resulting structure has higher activity than the original (unprocessed) enzyme. This is why Applicants refer to catalytic modification of the protein, without being bound by any theory. Thus, Applicants are reciting in certain claims that the change in structure, as evidenced by the change in CD/UV spectrum, is evidence of the efficacy of the claimed process, because this structural change is associated with an increase in activity, unlike in a conventional purification, where a change in structure leads to a decrease in activity.

On page 17 of the Office Action, the Examiner argues that:

> Applicant argues that the art does not teach or suggest the claimed process of contacting a diluted enzyme solution to activated carbon, in which the cells have been removed. Laustsen by themselves do teach such a process.

Laustsen absolutely does not teach or even suggest the claimed invention for the many reasons of record, which is further confirmed by the presently submitted Rule 132 Declaration discussed above that directly compares the claimed invention to Laustsen. Moreover, if Laustsen did teach the claimed method, Laustsen surely would have disclosed those method steps and claimed them because the claimed invention surprisingly results in a large reduction in the amount of required enzymes and corresponding reduction in costs associated with purchasing or producing the enzymes.

On page 17 of the Office Action, the Examiner argues that:

> In response to applicant previous argument dated 10/19/2006 p.[11] of the response, applicant had argued that they do not claim a specific CD spectra, but rather that there had been a change in structure as supported by CD spectra. The examiner's argument, "Applicant absolutely claims a specific CD spectra in claims 12-15, thus applicants arguments are not supported by the claims." Applicant now argues in the response filed 5/2/2207 that this statement is not understood and that claims 11-15 show changes in the defined spectra in the claims. Therefore, in claims 12, applicant **does claim a specific CD spectral range.**

This statement is not understood by Applicants. Reference to claims 11 to 15 listed below recite **changes in the defined spectra** as highlighted by Applicants' underlining in the claims. In other words, the spectra for the claimed enzyme solution is different from the conventional enzyme solution in the recited spectral range, which is very different from claiming a specific spectral range.

11. A method as defined in claim 1 wherein said enzyme solution of enhanced activity has a spectrum selected from Far UV (CD) and UV visible spectra *distinct* from said raw enzyme solution.

12. A method as defined in claim 11 wherein said enzyme solution of enhanced activity shows a relative absorbance intensity *lower* than

said raw enzyme solution, in the CD spectral range of 205-230 nm.

13. A method as defined in claim 11 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a Far UV (CD) spectrum minimum ellipticity *shifted* by at least 1 nm, from the raw enzyme solution, in the range between 205-230 nm.

14. A method as defined in claim 1 wherein said enzyme solution of enhanced activity has a UV-visible spectrum maximum peak at least 30 nm *lower* than said raw enzyme solution.

15. A method as defined in claim 1 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a maximum spectral absorption peak over the range 340 to 360 nm.

It is clear that these claims define a *change* in spectral properties, not an absolute spectrum as stated by the Examiner.

On pages 17-18 of the Office Action, the Examiner argues that:

> Applicant argues that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so. However, Laustsen does teach dilution prior to purification. In response to applicant's arguments that there is no motivation or teaching/suggestion, applicant is advised that KSR forecloses the argument that a specific teaching, suggestion, or motivation is required to support a finding of obviousness. See the recent Board decision *Ex parte* Smith,--USPQ2d--, slip op at 20,(Bd. Pat. App & Interf. June 25, 2007)(citing *KSR,82* USPQ2d at 1396) (available at http://www. uspto. govt we b/off ices/dcom/bpai/prec/fdO7 1925. pdf)

Applicant did not only argue "that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so" as alleged by the Examiner. Applicant also pointed out how mere dilution of Laustsen's method still does **not** result in the claimed invention. The Rule 132 Declaration submitted herewith comparing the claimed invention to the specific teachings in Laustsen confirms such.

Furthermore, this Rule 132 Declaration and the previous Rule 132 Declaration of record confirm the unexpected advantages of the claimed invention compared to the

prior art. Applicants are not required to compare their invention to a theoretical method that does not exist. Applicants are only required to compare their invention to what is known in the art, i.e. actual teachings, such as an experimental example. KSR specifically states that unexpected advantages overcome a prima facie case of obviousness. Thus, according to the holding in KSR, the Section 103 rejections should be withdrawn in view of the unexpected results confirmed by the experimental evidence of record in the originally filed application and Rule 132 Declarations of record.

On page 18 of the Office Action, the Examiner agues that:

> In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller,* 642 F.2d 413, 208 USPO 871 (COPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPO 375 (Fed. Cir. 1986).

Applicant has not attacked the references individually. In addressing the rejection based on the combination, Applicant has clearly pointed out what each reference in fact teaches (and fails to teach) and then shows how the combination of those teachings fails to make obvious the claimed invention.

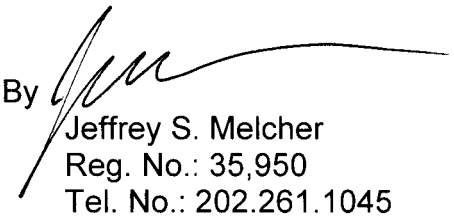
In view of the many differences between the claimed invention and the prior art and theoretical combination of references, and the unexpected advantages of the claimed invention, withdrawal of the Section 103 rejection is respectfully requested.

Applicants attach herewith a Form PTO/SB/08 listing the enclosed document entitled Activated Carbon. The Aikat reference cited by the Examiner is listed in the document. The document describes the known uses for activated carbon. Enhancement of enzyme activity is not disclosed as a known use for activated carbon. Page 12, lines 661-663 of this document teaches that "activated carbon catlyses the deamination (oxidation) of amino acids," which is destruction of the amino acids. Thus, this documents teaches to avoid using activated carbon with amino acids.

Consideration of this document and the Examiner's signature on the attached Form PTO/SB/O8 is respectfully requested. Please charge any required fees to have this Information Disclosure Statement entered to our deposit account No. 500687.

In view of all of the objections and rejections of record having been addressed, Applicants submit that the present application is in condition for allowance and Notice to that effect is respectfully, requested.

Respectfully submitted,
Manelli Denison & Selter, PLLC

By 

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